Peripherally administered CRF stimulates colonic motility via central CRF receptors and vagal pathways in conscious rats

Kiyoshi Tsukamoto, Yukio Nakade, Christopher Mantyh, Kirk Ludwig, Theodore N. Pappas, and Toku Takahashi

Department of Surgery, Duke University Medical Center, and Durham Veterans Affairs Medical Center, Durham, North Carolina

Submitted 6 October 2005; accepted in final form 3 November 2005

Peripheral administration of a CRF antagonist (16) demonstrates that defecatory response is antagonized by the central administration (DMV) in vivo and in vitro (11). Stress-induced stimulation of CRF stimulates the dorsal motor nucleus of the vagus nerve (9). Electrophysiological study demonstrated that administered CRF on colonic transit is abolished by truncal vagotony (10, 22). The stimulatory effect of centrally injected CRF stimulates colonic motor function by central CRF activity. However, it is controversial whether stress-induced stimulation of colonic motility is mediated via central or peripheral CRF receptors. We investigated the hypothesis that peripherally injected CRF accelerates colonic motility through the central CRF receptor, but not the peripheral CRF receptor. A strain gauge transducer was sutured on the serosal surface of the proximal colon. Colonic motility was monitored before and after the peripheral injection of CRF. An in vitro muscle strip study was also performed to investigate the peripheral effects of CRF. Subcutaneous injection of CRF (30–100 μg/kg) stimulated colonic motility in a dose-dependent manner. The stimulatory effect of peripherally administered CRF on colonic motility was abolished by intracisternal injection of astressin (a CRF receptor antagonist). No responses to CRF (10−9 – 10−7 M) of the muscle strips of the proximal colon were observed. These results suggest that the stimulatory effect of colonic motility in response to peripheral administration of CRF is mediated by the vagus nerve, nicotinic receptors, muscarinic receptors, and CRF receptors of the brain stem. It is concluded that peripherally administered CRF reaches the area postrema and activates the dorsal nucleus of vagi via central CRF receptors, resulting in stimulation of the vagal efferent and cholinergic transmission of the proximal colon.

There is accumulated evidence that stress stimulates the release of corticotropin releasing factor (CRF) from the paraventricular nucleus and that released CRF stimulates colonic motor activity. However, it is controversial whether CRF-induced acceleration of colonic motility is mediated via central CRF receptors (4) or peripheral CRF receptors (3). Stress or centrally injected CRF stimulates colonic motor function by cephalic CRF receptors (10, 22). The stimulatory effect of centrally administered CRF on colonic transit is abolished by truncal vagotony (9). Electrophysiological study demonstrated that CRF stimulates the dorsal motor nucleus of the vagus nerve (DMV) in vivo and in vitro (11). Stress-induced stimulation of defecatory response is antagonized by the central administration of a CRF antagonist, but not by the peripheral administration of a CRF antagonist (16). These reports indicated that centrally released CRF induced by stress stimulates vagal efferent, resulting in stimulation of colonic motility and transit.

In contrast, others showed that peripherally administered CRF stimulates colonic motility via its own peripheral receptors, and the stimulatory effect of CRF is antagonized by peripheral injection of CRF antagonists (13, 14, 26). Restraint stress-induced stimulation of fecal pellet output is antagonized by the peripheral administration of a CRF antagonist (26). CRF-producing cells are present in the colonic mucosa (7, 19). It is proposed that stress may stimulate the release of CRF from the colonic mucosal cells and that released CRF contracts colonic muscle via CRF receptors of the myenteric plexus (3, 23).

Our recent study demonstrated that restraint stress-induced acceleration of colonic transit is abolished by the central injection of astressin, but not by the peripheral injection of astressin (20). We hypothesize that peripherally administered CRF stimulates colonic motility through central CRF receptors, but not peripheral CRF receptors. We studied motility of the proximal colon by using strain gauge transducers in conscious rats and investigated the effects of truncal vagotomy, hexamethonium, atropine, and intracisternal injection of astressin (3, 21, 23). It is proposed that stress may stimulate the release of CRF from the colonic mucosal cells and that released CRF contracts colonic muscle via CRF receptors of the myenteric plexus.

**MATERIALS AND METHODS**

**Animal preparations.** All procedures used in this study were approved by the Durham Veterans Affairs Medical Center (Durham, NC). Male Sprague-Dawley rats weighing 280–330 g were used and fed with laboratory rodent chow and water ad libitum. All surgical procedures were carried out with the animals under isoflurane anesthesia.

A strain gauge transducer was sutured on the proximal colon parallel with circular muscle to record its contractions. Wires from a strain gauge transducer were put through a subcutaneous tunnel and out the dorsal. Five days after operation, the colonic motility study was performed.

**Recording of colonic motility.** Rats were placed in a cage and wires from strain gauge transducers were connected to the recording system (Power Lab/4SP, AD Instruments, Colorado Springs, CO). The motility recordings were performed at least 3 h before and after the administration of CRF or saline. The area under the curve of the motility recording was measured as a motility index (MI) by using a computer-assisted system (Power Lab) as previously reported (24).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
expressed as 100% (control), and the MI after the injection of CRF or saline for 1 h was expressed as a percentage of the control.

Effect of peripheral CRF on colonic motility. To determine the effective dose of peripheral CRF on colonic motility, the colonic motility was measured with subcutaneous injection of CRF. In this study, several doses (3, 10, 30, and 100 μg/kg) of CRF were used. Each dose of CRF was diluted by saline (100 μl). Saline (100 μl sc) was used as a control.

To study whether the vagus nerve is related to the alteration of colonic motility evoked by CRF, subdiaphragmatic bilateral truncal vagotomy was performed during the operation to install the strain gauge transducers. Five days after the operation, the colonic motility was recorded before and after CRF injection (30 μg/kg sc).

To investigate whether nicotinic or muscarinic receptors are involved in mediating CRF-induced stimulation of colonic motility, hexamethonium (20 mg/kg sc) or atropine (200 μg/kg sc) was injected 20 min before the injection of CRF (30 μg/kg sc).

In vitro muscle strip study. To investigate whether peripheral CRF receptors located on the myenteric plexus mediate colonic contraction in response to CRF, we investigated by using an in vitro organ bath study. Rats were fasted overnight and anesthetized by xylazine (13 mg/kg) and ketamine (87 mg/kg) anesthesia. Circular muscle strips were isolated from the proximal colon. As previously described (25, 27), circular muscle strips (10 × 3 mm) obtained from the proximal colon were suspended between two platinum electrodes in a 30-ml organ bath filled with Krebs-Henseleit buffer containing 0.1% BSA.

Fig. 1. Representative tracing of subcutaneous injection of saline (a) and corticotropin releasing factor (CRF; 3–100 μg/kg; b–e)-induced motility of the proximal colon in conscious rats (A). Percentage of motility index (MI) change in response to saline and CRF (B). CRF increased colonic motility in a dose-dependent manner (30–100 μg/kg). The smaller doses of CRF (3 and 10 μg/kg) exhibit a slight but not a significant increase in colonic motility, whereas the higher doses of CRF (30 and 100 μg/kg) caused a significant increase of colonic motility (n = 4–5; *P < 0.05).
Mechanical activity was recorded on a polygraph using isometric transducers. Effects of different doses of CRF (10^{-9} – 10^{-7} M) on colonic motility were investigated. Colonic motor responses to carbachol (10^{-5} M) and transmural nerve stimulation (65 V, 10 Hz, 0.5 ms) were also performed.

Statistical analysis. Results were expressed as means ± SE. The data were evaluated by Student’s t-test, paired t-test, or repeated-measure ANOVA followed by Dunnett’s test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

The smaller doses of subcutaneous injection of CRF (3 and 10 µg/kg) exhibited a slight, but not a significant, increase of colonic motility, whereas the higher doses of CRF (30 and 100 µg/kg) caused a significant increase of colonic motility ($P < 0.05$) (Fig. 1).

The stimulatory effect of CRF (30 µg/kg sc) on colonic motility was abolished by the premedication with hexamethonium (20 mg/kg sc) and atropine (200 µg/kg sc) ($P < 0.05$) (Fig. 2).

CRF (30 µg/kg sc) had no more stimulatory effects in rats treated with truncal vagotomy. In contrast, CRF (30 µg/kg sc) caused colonic contractions in rats treated with sham vagotomy (Fig. 2).

Following intracisternal injection of astressin (10 µg), the stimulatory effect of CRF (30 µg/kg sc) was significantly attenuated to 114 ± 29% of MI change ($n = 5$), compared with that of saline-injected rats (181 ± 35% of MI change) ($n = 4$, $P < 0.05$) (Fig. 3).

In an in vitro muscle strip study, carbachol (10^{-5} M) and transmural nerve stimulation (65 V, 10 Hz, 0.5 ms) caused muscular contractions in vitro, suggesting that muscarinic receptors and cholinergic transmission are intact in the neuromuscular preparations of the proximal colon. In contrast, no muscle contractions were observed following the administration of CRF (10^{-9} – 10^{-7} M) (Fig. 4).

DISCUSSION

One of the most frequent major clinical symptoms of irritable bowel syndrome (IBS) is functional bowel disorder. It is well known that stress is one of the most frequent contributing factors to the pathogenesis of bowel disorder in IBS patients (1,
CRF plays a key role in stress-induced alteration of colonic motility (26).

It is controversial whether stress-induced acceleration of colonic motility is mediated via peripheral CRF receptors (3) or central CRF receptors (4). Intracerebroventricular injection of CRF (1, 3, and 10 μg) dose dependently increases the number of fecal pellet output, whereas intravenous injection of CRF (up to 100 μg/kg) does not affect defecation in rats (18). The stimulatory effect of restraint stress on colonic transit is abolished by intracerebroventricular injection of a CRF antagonist (10). The stimulatory effect of intracerebroventricular injection of CRF on colonic transit is abolished by truncal vagotomy in rats (9). An in vivo and in vitro electrophysiological study revealed that CRF stimulates, directly or indirectly, DMV via the nucleus of the tractus solitarius in rats (11). These results suggest that CRF released by stress acts as its own receptors at the CNS, resulting in stimulation of colonic motility. We have recently shown that restraint stress-induced acceleration of colonic transit is abolished by the central injection of astressin, but not by the peripheral injection of astressin (20).

In contrast, others have suggested the possibility that the stimulatory effect of CRF on colonic motility is mediated via peripheral CRF receptors. Stress-induced acceleration of colonic transit is antagonized by peripheral administration of a CRF antagonist (13, 14, 26). These reports are inconsistent with our results. It is possible that the receptor distribution is diverse because of the different genetic phenotypes of the animals developed by the different vendors.

Our study revealed that the stimulatory effect of peripherally administered CRF on colonic motility was abolished by hexamethonium and atropine. Furthermore, truncal vagotomy also abolished CRF-stimulated colonic contractions. These findings indicated that peripheral CRF-induced stimulation of colonic motility is mediated via vagal cholinergic pathways. The intracisternal injection of astressin also abolished the stimulatory effect of CRF (30 μg/kg sc), suggesting that the stimulatory effect of peripheral CRF is mediated via central CRF receptors. It is conceivable that peripheral administration of CRF activates the DMV and vagal efferent via central CRF receptors probably at the circumventricular organs that are relatively unprotected by the blood-brain barrier (5).

CRF type 1 receptors are expressed in the goblet and stem cells of the crypts and in scattered cells of the surface epithelium and the lamina propria of the colonic mucosa. CRF type 1 receptors are also expressed in the submucosal plexus and myenteric plexus of the rat colon (3).

However, it is still controversial whether the effect of CRF on the colonic smooth muscle in vitro strip is inhibitory or excitatory. CRF (10^{-10} − 10^{-8} M) provokes a concentration-dependent increase of mechanical activity of the isolated colon in vitro in rats (15). CRF causes c-fos expression on the cholinergic neurons of the colonic myenteric plexus (17). In contrast, the effect of CRF (10^{-13} − 10^{-7} M) on colonic smooth muscle cells of guinea pig has been shown to be inhibitory, not excitatory, in vitro (6). In our in vitro muscle strip study, CRF (10^{-9} − 10^{-7} M) had no stimulatory effects on colonic motility. This indicates that CRF receptors expressed in the myenteric plexus are not involved in mediating muscular contractions of the proximal colon in rats. CRF has been shown to stimulate mucosal ion secretion (23) and increases mucin release of the rat colon (2).

The expression of CRF type 1 receptor mRNA is also observed in both myenteric and submucosal plexuses of the guinea pig colon. Application of CRF (10^{-9} M − 3 × 10^{-7} M) evokes slowly activating depolarizing responses associated with elevated excitability in both myenteric and submucosal neurons of the guinea pig colon in vitro (12). However, there are no data available showing whether CRF causes muscular contraction of the guinea pig colon in vitro. It is also not known whether restraint stress and CRF (central and peripheral) stimulates colonic motility of guinea pigs. Thus it remains unclear whether CRF type 1 receptor located in the myenteric plexus is responsible for muscular contraction of the guinea pig colon.

In summary, the result of our current study indicates that peripheral CRF stimulates vagal efferent via central CRF receptors, resulting in activation of cholinergic transmission of...
the myenteric plexus, and causes muscle contractions of the rat proximal colon.

ACKNOWLEDGEMENT

This study was supported, in part, by National Institute of Diabetes and Digestive and Kidney Diseases Grants RO1-DK-55808 and RO1-DK-62768 (to T. Takahashi).

REFERENCES


